Bcl-2 Antisense Oligonucleotides (G3139) Inhibit Merkel Cell Carcinoma Growth in SCID Mice

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Merkel cell carcinoma was first described in 1972 by Toker and is an aggressive neuroendocrine skin tumor with a high metastatic potential. Merkel cell carcinoma is thought to derive from the neuroendocrine (Merkel) cells of the skin, although in contrast to fetal and especially adult Merkel cells, Merkel cell carcinomas express high levels of the Bcl-2 oncoprotein. Bcl-2 is capable of blocking programmed cell death and has been shown to play an important role in normal cell turnover, tumor biology, and chemoresistance. High Bcl-2 expression leading to prolonged survival of cells may therefore be of importance in the biological and clinical characteristics of Merkel cell carcinoma. In a SCID mouse xenotransplantation model for human Merkel cell carcinoma, we investigated the influence of the *bcl-2* antisense oligonucleotide G3139 (Genta) on tumor growth in comparison with control oligonucleotides or cisplatin. *Bcl-2* antisense treatment, targeting the first six codons of the *bcl-2* mRNA, resulted in either a dramatic reduction of tumor growth or complete remission, whereas reverse sequence and two-base mismatch control oligonucleotides or cisplatin had no significant antitumor effects compared with saline-treated controls. Apoptosis was enhanced 2.4-fold in the *bcl-2* antisense treated tumors compared with the saline-treated group, and no other treatment showed a comparable increase in apoptosis. Our findings suggest that *bcl-2* antisense treatment may be a novel approach to improve treatment outcome of human Merkel cell carcinoma. *Key words: apoptosis/tumor therapy/xenotransplantation model. J Invest Dermatol 114:725–730, 2000*

Merkel cell carcinoma (MCC) is an unusual malignant neuroendocrine tumor with aggressive growth behavior that may be difficult to diagnose and treat effectively, particularly in advanced stages of the disease (Ratner *et al*, 1993). This tumor typically occurs in persons older than 65 y of age, but patients ranging in age from 7 to 95 y have been reported. The etiology of MCC is unknown, but it is interesting to note that the tumor occurs most frequently on sun-exposed sites. The most common location of MCC is the skin of head and neck with about 50% of all tumors arising in this area, followed by the extremities, buttocks, and trunk. MCC has a high incidence of local recurrence, and regional as well as systemic spread. Approximately one-third of patients have a local recurrence within 1 y of excision, one-half to two-thirds of patients develop regional lymph node metastases, and distant metastases are found in more than one-third of patients (O'Connor and Brodland, 1996). Like malignant melanoma MCC spreads primarily via the lymphatics to regional lymph nodes and the prognosis for regional and systemic metastasis is similar. One, 2 and 3 y survival rates have been estimated at 88%, 72%, and 55%, respectively (Hitchcock *et al*, 1988).

Patients are usually staged based on their clinical presentation. Stage I disease presents with a primary tumor and no evidence of regional nodal involvement, stage II with regional nodal involvement, and stage III disease is defined as the presence of systemic metastases (Yiengpruksawan *et al*, 1991). For localized disease, wide surgical excision is the treatment of choice in most cases of MCC, but additional irradiation of the primary site after excision as well as the regional draining lymph node basin has been recommended (Haag *et al*, 1995). For regional lymph node metastases, irradiation may be used in conjunction with nodal dissection, and for regional metastatic disease the use of systemic chemotherapy has also been suggested. For disseminated disease, chemotherapy is the most often employed treatment. Combinations of chemotherapeutic agents that are also used for small cell lung cancer or cisplatin-containing regimens have been used with variable success (Sharma *et al*, 1991; Pectasides *et al*, 1995). Similar to small cell lung cancer, high rates of initial response have been reported after chemotherapy for MCC, but such responses are not commonly durable (Haag *et al*, 1995).

Recent immunohistochemical studies have investigated the expression of the *bcl-2* oncogene in MCC (Kennedy *et al*, 1996; Moll *et al*, 1996; Plettenberg *et al*, 1996) and a high expression of

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Abbreviation: MCC, Merkel cell carconoma.

Bcl-2 in MCC compared with fetal and adult Merkel cells was reported (Moll *et al*, 1996). The Bcl-2 protein is capable of blocking apoptosis, which is not only an important mechanism in the maintenance of tissue homeostasis but also the mechanism of action of several chemotherapeutic drugs (Hockenbery, 1994; Reed, 1994). As high Bcl-2 protein levels in MCC might rescue the transformed cells from apoptosis and thereby contribute to tumor growth of this type of malignancy, modulation of Bcl-2 expression may offer a new strategy in the therapy of MCC.

Antisense oligonucleotides hold great promise as agents for the specific manipulation of gene expression. Modified analogs, particularly phosphorothioates, have been used to specifically inhibit gene expression both *in vitro* and *in vivo* (Neckers *et al*, 1992; Wagner, 1995; Askari and McDonnell, 1996). Downregulation of Bcl-2 expression by antisense oligonucleotides has been reported to make leukemic cells more susceptible to apoptosis (Campos *et al*, 1994) and to suppress B cell lymphoma growth in SCID mice (Cotter *et al*, 1994). G3139, a phosphorothioate oligonucleotide targeted to the first six codons of the *bcl-2* mRNA, is a drug that is now in clinical trials (Banerjee, 1999). Downregulation of Bcl-2 protein expression *in vivo* has been reported using G3139 treatment in a murine tumor model (Jansen *et al*, 1998) and in early results from a clinical trial (Webb *et al*, 1997).

In this study we investigate the influence of *bcl-2* antisense oligonucleotides on the growth characteristics of human MCC in a SCID-hu xenotransplantation model.

MATERIALS AND METHODS

Reagents Phosphorothioate oligonucleotides corresponding to the human *bcl-2* translation initiation site were obtained from Genta (San Diego, CA). The antisense and reverse control sequences were 5′Tctcccagcgtgcgccat3′ (G3139) and 5′Taccgcgtgcgaccctct3′ (G3622), respectively. A two-base *bcl-2* antisense mismatch oligonucleotide 5′Tctcccagcatgtgccat3′ (G4126) was used as an additional control.

Cell culture The human MCC line MC-MA 11 (Moll *et al*, 1994; Jansen *et al*, 1999), established from the primary tumor of a 91-y-old male patient, was cultured on a feeder layer of lethally irradiated adult human dermal fibroblasts in RPMI 1640 (Gibco BRL, Paisley, U.K.) supplemented with 15% fetal bovine serum (Gibco), 10 μg per ml transferrin (Sigma, St Louis, MO), 5 μg per ml insulin (Gibco), and 2 mM L-Glutamin (Gibco) in a humidified 5% CO_2–95% ambient air atmosphere at 37°C.

Experimental animals, generation of tumors, and therapy Pathogen-free female C.B-17 *scid/scid* (SCID) mice (Bosma *et al*, 1983), 4–6 wk old, tested for leakiness, were obtained from Bomholtgard Breeding and Research Center (Ry, Denmark). The animals were housed in laminar flow racks and microisolator cages under specific pathogen-free conditions and received autoclaved food and water but no antibiotic prophylaxis. All procedures involving animals and their care were in accordance with institutional and government guidelines. For the *in vivo* experiment, cells were serially passaged by three consecutive transplantations prior to the start of treatment. Tumor take in these experiments was always 100%, but consecutive passaging resulted in less variability in the actual tumor size. In brief, tumors were explanted under sterile conditions, dissected, and 1×10^7 viable MCC cells of the dissected tumor were injected again subcutaneously in SCID mice. For the antisense experiment SCID mice were injected subcutaneously into the left lower flank with 1×10^7 human MC-MA 11 MCC cells and after 3 wk, when all animals had developed palpable tumors, were randomly assigned to five treatment groups (n = 6 per group). An additional group of animals (n = 3) was sacrificed the same day to determine the tumor weight at the initiation of treatment. Mice in the oligonucleotide groups or the saline control group were implanted with prefilled miniosmotic pumps (pumping rate 0.25 μl per hour, 28 d; Alzet model 2004, Alza, Palo Alto, CA) into an interscapular pocket and treated with *bcl-2* antisense (group A), *bcl-2* reverse control (group B), *bcl-2* mismatch (group C), or saline (group D). An oligonucleotide dosage of 10 mg per kg per d was administered over a period of 4 wk. The mice in group E were treated with cisplatin three times intraperitoneally at 5 mg per kg every 4 d. Twenty-eight days after initiation of treatment, all animals were sacrificed and evaluated for tumor growth and organ involvement.

Western blot analysis Frozen tumor samples were homogenized in liquid nitrogen and extracted in a buffer containing 0.14 M NaCl, 0.2 M triethanolamine, 0.5% NP-40, and 0.2% sodium deoxycholate (pH = 7.4) supplemented with protease inhibitors (4 μg per ml aprotinin, 4 μg per ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride). Soluble protein was quantified using a modified Bradford analysis (Bio-Rad, Richmond, CA) and the extracts were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (12%), blotted onto PVDF membranes (Millipore), and analyzed using a mouse monoclonal antibody specific for human Bcl-2 (Santa Cruz Biotechnology, Santa Cruz, CA) and a rabbit polyclonal antiactin antibody (Sigma, Milwaukee, WI). Alkaline phosphatase-coupled goat antimouse IgG or goat antirabbit antibodies (Tropix, Bedford, MA) were used in secondary incubations followed by detection of the reactive bands by chemiluminescence substrate (CSPD substrate, Tropix). The expression levels of Bcl-2 and actin protein were quantified by densitometry of autoradiograms with a Herolab E.A.S.Y. RH densitometer (Herolab, Wiesloch, Germany) and the E.A.S.Y Win32 (Herolab) software. Signal strength of each Bcl-2 signal was normalized to actin and the ratios between Bcl-2 protein in control tumor extracts and oligonucleotide-treated extracts were calculated. Serial dilutions of control tumor extracts were included in each analysis to ensure that the signal strength of the test bands was in the linear range of the scanner.

Apoptosis analysis of MCC Human MC-MA 11 MCC grown in SCID mice were fixed in 7.5% neutral buffered formalin, dehydrated, and embedded in paraffin by routine methods. Five micron sections were prepared, dewaxed in xylene, and rehydrated through a graded series of ethanol and distilled water. The tissue sections were incubated with proteinase K and the immunohistochemical detection of apoptosis was performed by TUNEL staining (Böhringer Mannheim, Mannheim, Germany). Terminal deoxynucleotidyl transferase labeling with fluorescein-dUTP was done according to the manufacturer's recommendations and the tissue sections were counterstained with 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI; Böhringer Mannheim). Four randomly chosen tumors per group were evaluated for the presence of apoptotic cells. Cells were counted as apoptotic when the whole nuclear area of the cell labeled positively or when apoptotic bodies (small positively labeled globular bodies in the cytoplasm of the tumor cells, either single or in groups) were present in the cell. Necrotic areas were present in the larger tumors and apoptotic cells were often identified in the vicinity of necrotic zones, but such areas were not included in assessing the apoptotic index. To estimate the apoptotic index for each individual tumor (percentage of apoptotic cells in a given tumor) apoptotic cells in four randomly selected fields of the TUNEL stained tumor section or, for the smaller tumors, in four fields of nonserial sections, were counted. Depending on the tissue sections investigated, these four fields contained 5000–6000 cells. The investigator scoring apoptotic cells was blind as to the treatment groups. The apoptotic index for each individual tumor was calculated as a percentage of TUNEL-positive cells using the following formula: apoptotic index = (number of TUNEL-positive cells/number of total cell nuclei counted) × 100. The mean apoptotic index for each treatment group was determined as the sum of apoptotic indices of individual tumors divided by the number of tumors investigated.

Statistical analysis The statistical significance of differences in tumor weight between treated animals in the respective groups and between the apoptotic indices of the evaluated tumors was determined using the Mann–Whitney U test. p-values of less than 0.05 were considered to be of statistical significance.

RESULTS

Influence of different treatments on tumor growth and animal health SCID mice were injected subcutaneously into the left lower flank with 10^7 MC-MA 11 cells and after 3 wk, when all animals had developed palpable tumors (mean tumor weight 0.14 ± 0.02 g SD, determined in an additional group as described in the *Materials and Methods*), were randomly assigned to treatment groups. Miniosmotic pumps filled with *bcl-2* antisense, *bcl-2* reverse control, or *bcl-2* mismatch oligonucleotides, or saline alone, were implanted into a small interscapular pocket and the oligonucleotides were released at a dosage of 10 mg per kg per d over a period of 4 wk. During the treatment period animals were monitored daily for changes in mobility, food intake, and fur appearance. Local tumor growth was estimated weekly and a growth reduction was apparent in the antisense group after 2 wk of continuous infusion, becoming

even more pronounced after 3 wk. After 4 wk of continuous infusion of oligonucleotides or saline, all animals were sacrificed and evaluated for tumor growth. Marked differences were found in the mean tumor weight (**Fig 1**) between SCID mice with established MC-MA 11 human MCC treated with *bcl-2* antisense oligonucleotides and those treated with saline, reverse control, or mismatch control oligonucleotides. In the antisense group a significant reduction of tumor growth or even complete ablation (one out of six animals) was observed (mean tumor weight 0.29 g ± 0.14 g SD, $p < 0.05$). In contrast, saline, reverse control, or mismatch oligonucleotides showed no such effect (mean tumor weight ± SD, 2.30 ± 0.47 g, 2.19 ± 0.77 g, 2.24 ± 0.34 g, respectively).

Cisplatin or cisplatin-based regimens are used for the treatment of MCC. In an additional group, six mice were injected iintraperitoneally with cisplatin at a dosage of 5 mg per kg three times every 4 d. The experiment was terminated 28 d after the initiation of treatment and cisplatin treatment had no significant influence on tumor growth compared with saline-treated controls. The mean tumor weight in the cisplatin-treated group was 2.22 ± 0.46 g SD (**Fig 1**).

At the end of the 4 wk treatment period some animals in all groups except the antisense group were hampered in their mobility due to local tumor load. Mouse weight was evaluated after termination of the experiment (**Fig 2**). Oligonucleotide treatment at a dosage of 10 mg per kg per d was well tolerated by the mice, leading to no weight loss compared with the saline-treated controls (mean mouse weight ± SD: 22.2 ± 1.8 g saline, 21.9 ± 1.4 g reverse control, and 22.3 ± 1.2 g mismatch control). Animals in the antisense group, having no or only minimal tumor burden, presented with a larger mouse weight at the end of the experiment (mean mouse weight ± SD: 25.5 ± 0.5 g). Cisplatin led to a weight loss of about 10% (mean mouse weight ± SD: 20.1 ± 0.8 g) compared with saline-treated controls, an effect described when using high doses of chemotherapeutic agents in the SCID mouse (Paine-Murrieta *et al*, 1997).

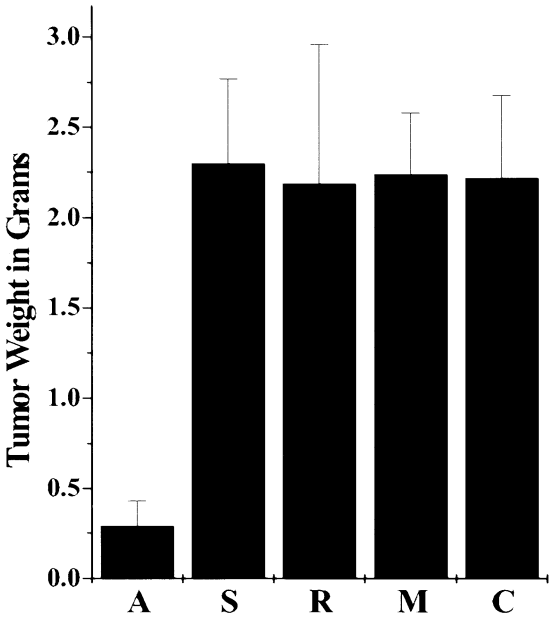

Figure 1. Growth inhibition of human MCC by bcl-2 antisense oligonucleotides. SCID mice (n = 6 per group) injected subcutaneously with 1×10^7 human MCC cells received a 28 d continuous infusion by osmotic pumps of saline (S), *bcl-2* antisense (A), reverse control (R), or mismatch (M) oligonucleotides starting 3 wk after tumor cell inoculation. A dosage of 10 mg per kg per d of oligonucleotides was administered. Alternatively animals were treated with cisplatin (C) administered intraperitoneally three times at a dosage of 5 mg per kg every 4 d. Animals in all groups were evaluated 4 wk after initiation of treatment. Mean tumor weights in grams ± SD of the respective groups are shown.

Reduction of Bcl-2 levels by antisense treatment Western blot analysis of oligonucleotide-treated tumors with a human specific anti-Bcl-2 antibody showed a reduction of Bcl-2 gene product in the antisense group compared with saline-treated controls, whereas reverse control or mismatch oligonucleotides had no such effect. Mean Bcl-2 levels compared with controls of four independent experiments were 0.69 ± 0.1 SD (antisense), 1.04 ± 0.09 SD (reverse control), and 1.17 ± 0.2 SD (mismatch control). **Figure 3** shows a representative example of a western blot of Bcl-2 (**Fig 3*A***) and actin (**Fig 3*B***) and the respective densitometry is given in **Fig 3** (***C, D***).

Influence of treatment on the number of apoptotic cells in MCC Five micron sections of formalin fixed tumor samples were prepared and apoptotic tumor cells were labeled with fluorescein-dUTP (TUNEL Assay; **Fig 4**). Sections were additionally stained with DAPI to visualize also nonapoptotic nuclei. Bcl-2 antisense treatment significantly increased the mean apoptotic index (4.6% ± 1.3% SD; $p < 0.05$) within sections of human MCC grown in SCID mice compared with saline, reverse control, or mismatch oligonucleotide treated mice (mean apoptotic index ± SD: 1.9% ± 0.8% saline, 1.6% ± 0.6% reverse, and 2.0% ± 0.9% in the mismatch group, respectively). In tumor sections of cisplatin-treated mice very few apoptotic cells were detected (mean apoptotic index ± SD: 0.4% ± 0.2%).

DISCUSSION

Current treatment of advanced MCC is based on a combination of surgery, radiotherapy, and/or chemotherapy. MCC often responds initially to chemotherapy, but this response is frequently not lasting. Additional limitations for chemotherapeutic intervention lie in the low tolerability of agents at efficacious doses. As MCC affects mainly older people, problems of tolerability become even more important. Furthermore, currently employed chemotherapeutic agents, which act as general transcriptional inhibitors or DNA-damaging agents, lack specificity in their mechanism of action. Therefore, novel therapeutic strategies focusing on disease-relevant molecular targets are clearly warranted.

The *bcl-2* gene was originally identified in follicular lymphomas in association with the t(14; 18) translocation (Tsujimoto and Croce, 1986) and is thought to promote cell survival by inhibiting

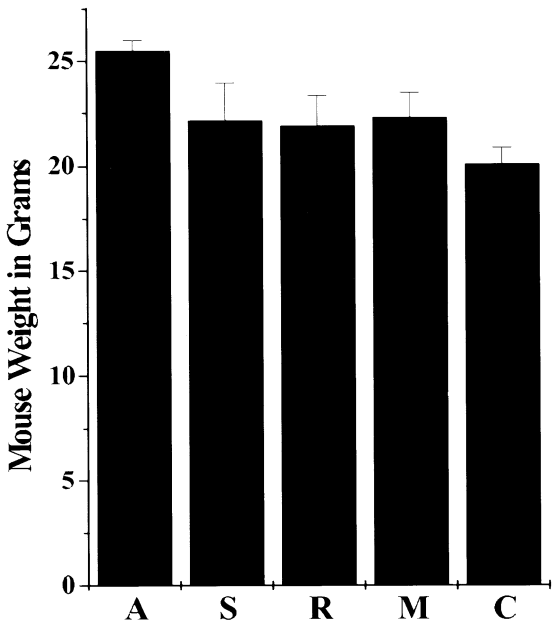

Figure 2. Influence of treatment on mouse weight. The mean mouse weight of the different treatment groups at the end of the experiment in grams ± SD is shown. (A, antisense; S, saline; R, reverse control; M, mismatch control; C, cisplatin.)

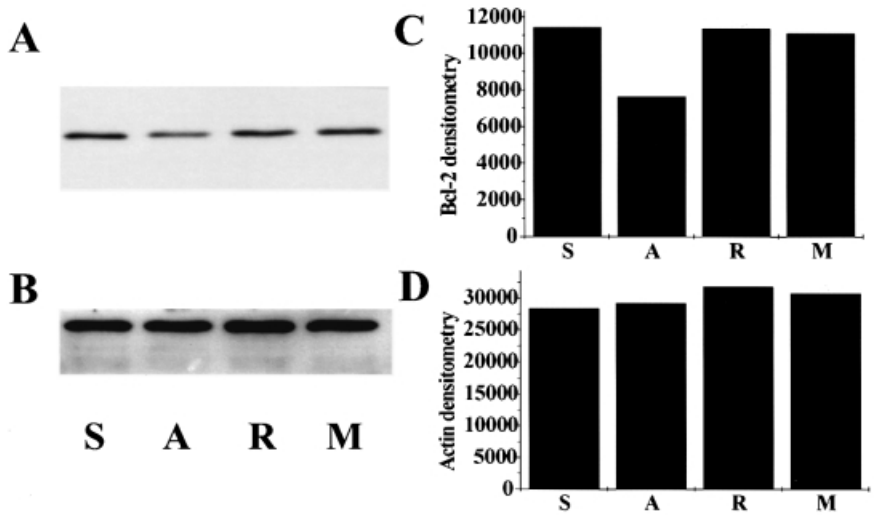

Figure 3. Bcl-2 protein levels of MCC Expression of Bcl-2 (*A*) and actin (*B*) in MCC grown in SCID mice. Ten micrograms total protein of saline (S), antisense (A), reverse control (R), or mismatch control (M) treated tumor extracts were loaded per lane. The blot was cut and incubated with anti-Bcl-2 (*A*) or antiactin (*B*) antibodies. The densitometry (expressed as arbitrary units) of the Bcl-2 blot is shown in (*C*) and of actin in (*D*).

programmed cell death (Hockenbery, 1994; Reed, 1994). The expression of Bcl-2 in Merkel cells could help cells overcome apoptotic stimuli in their physiologic environment, but might also contribute to the development of MCC by rescuing transformed Merkel cells from apoptosis. This notion is indirectly supported by the finding that Bcl-2 protein expression appears to be enhanced in MCC compared with Merkel cells (Moll *et al*, 1996), suggesting that Bcl-2 expression is essential for the development and growth of MCC. Downregulation of Bcl-2 expression with antisense oligonucleotides represents a method to modulate the expression of this protein in MCC and to study its influence on tumor growth.

In contrast to their putative cells of origin, the Merkel cells, MCC can be grown *in vitro*. MC-MA 11 cells grow in supplemented RPMI medium on human irradiated fibroblast feeder layers as loosely arranged aggregates. *In vitro* oligonucleotide treatment, often requiring the use of uptake-enhancing agents, is likely to interfere with the adhesion to the feeder layer (Khaled *et al*, 1996) and the feeder layer cells themselves, thereby causing growth inhibitory effects apart from a real antisense mechanism. Therefore, in an attempt to avoid such artifacts we tested the influence of *bcl-2* antisense oligodeoxynucleotide treatment on MCC growth *in vivo* using a SCID mouse xenotransplantation model.

Preclinical studies showed good tolerance of *bcl-2* antisense oligonucleotides at a dosage of 10 mg per kg (Banerjee, 1999) and treatment of established MCC with *bcl-2* antisense, but not with reverse control or two-base mismatch *bcl-2* antisense oligodeoxynucleotides, resulted in a reduction of tumor growth of 87% compared with saline-treated control tumors. Neither reverse control or mismatch control oligonucleotides had a significant effect on tumor growth (reductions of 5% or less). Oligonucleotide treatment was well tolerated by the mice, leading to no abnormalities other than splenomegaly and increased hematopoietic cell proliferation in the liver, well described class effects of phosphorothioates in rodents (Monteith *et al*, 1997).

MCC resembles small cell lung carcinoma and most chemotherapeutic agents selected for the treatment of MCC are also used in the treatment of small cell lung carcinoma. Cisplatin-containing regimens are used among others (Sharma *et al*, 1991) and sensitivity of MCC to cisplatin *in vitro* has been reported (Krasagakis *et al*, 1997). A cisplatin treatment regimen of 5 mg per kg injected three times intraperitoneally is well tolerated by SCID mice and caused a significant growth reduction of human melanomas not harboring an N-*ras* mutation in SCID mice (Jansen *et al*, 1997). A lower dosage (4 mg per kg three times intraperitoneally every 4 d) is reported to inhibit the growth of cisplatin-sensitive NCI-H69 small cell lung cancer tumors in SCID mice (Heike *et al*, 1995).

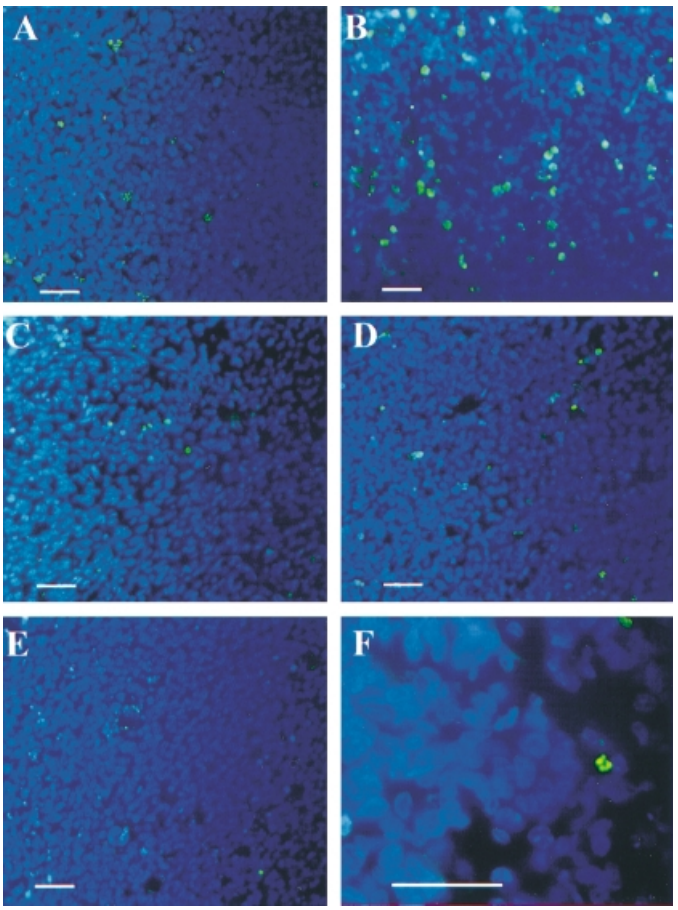

Figure 4. TUNEL staining of apoptotic cells in human MCC Five micromolar sections of formalin fixed tumor samples were incubated with terminal deoxynucleotidyl transferase and fluorescein-dUTP and counterstained with DAPI. Photographs of MC-MA 11 tumors treated with saline (*A*) or antisense (*B*), or reverse control (*C*) or mismatch (*D*) control oligonucleotides, or cisplatin (*E*) are shown. Part (*F*) shows a fluorescein isothiocyanate-labeled cell, fragmented into apoptotic bodies in an antisense-treated tumor. *Scale bars*: (*A–E*) 50 μm; (*F*) 45 μm.

Treatment of MC-MA 11 cells in SCID mice with cisplatin (5 mg per kg, intraperitoneally, administered three times every 4 d), however, caused no reduction of tumor growth.

Establishing that an antisense oligodeoxynucleotide acts through a sequence-specific mechanism is crucial, and usually the sequence-specific effect is inferred from a reduction of target messenger RNA or protein. Western blot analysis of oligodeoxynucleotide-treated tumors showed a reduction of Bcl-2 levels of about 30% in the antisense group, which was not observed in any other treatment group. This is a rather small reduction of Bcl-2 levels; after the second week of treatment, however, a tumor growth reduction was evident in the antisense group, and it might well be that after 4 wk of treatment only the more resistant tumor cells, still expressing high Bcl-2 levels, remained. The antiapoptotic Bcl-2 protein belongs to a growing family of Bcl-2 related proteins that can either inhibit or promote apoptosis. It is currently believed that the ratio of death antagonists (like Bcl-2, Bcl-X_L, Bcl-w, etc.) to agonists (Bax, Bak, Bad, Bid, etc.) determines whether a cell will respond to an apoptotic signal (Kroemer, 1997). Treatment of human MCC in SCID mice with G3139 reduced the Bcl-2 level in the tumor cells, but had no influence on the proapoptotic Bax protein (data not shown). It may well be that in susceptible cells even a slight change in the ratio of proapoptotic and antiapoptotic Bcl-2 family members results in a continuous increase in the numbers of apoptotic events, leading to dramatic differences in tumor weight after a 4 wk treatment period.

Histologically, MCC is characterized by prominent numbers of mitoses and apoptosis (Kennedy *et al*, 1996) and evaluation of the mean apoptotic index in control tumor sections of MC-MA 11 cells grown in SCID mice revealed 1.9% of naturally occurring apoptotic cells. *Bcl-2* antisense treatment enhanced the mean apoptotic index by about 2.4-fold to 4.6%. Control oligodeoxynucleotide treatment (reverse control or *bcl-2* two-base mismatch) caused no significant changes in the number of apoptotic cells (mean apoptotic index 1.6% and 2.0%, respectively). Only very few apoptotic cells could be detected in the cisplatin-treated group (mean apoptotic index 0.4%). This small percentage of apoptotic cells can be explained by the fact that the evaluation was performed more than 2 wk after the last cisplatin injection and that susceptible cells may have died earlier from apoptosis.

The sequence specificity of the effects observed in MCC argues for an antisense mechanism of action of the *bcl-2* antisense oligonucleotide used, although additional non-antisense interactions cannot be excluded (Stein, 1995). Unmethylated CpG motifs in phosphorothioate oligodeoxynucleotides are known to stimulate B cell proliferation and activate natural killer cells (Ballas *et al*, 1996). G3139, the antisense oligonucleotide used in this study, contains two CpG motifs and thus could potentially activate natural killer cells under some experimental conditions (Wooldridge *et al*, 1997). The reverse control oligonucleotide used in our experiment, however, also contains such motifs, and did not produce antitumor responses in our MCC model.

Phosphorothioate oligodeoxynucleotides represent an opportunity to specifically affect disease-relevant molecular targets and, in contrast to many chemotherapeutic agents used at present, have a relatively large therapeutic index. A phase I dose escalation trial (Webb *et al*, 1997) with G3139 in lymphoma patients demonstrated that this phosphorothioate oligodeoxynucleotide is well tolerated in humans and improvements in the biochemical and hematologic variables, as well as tumor regression and downregulation of Bcl-2 protein expression, were reported. A phase I/II study for malignant melanoma with G3139 in combination with dacarbazine is currently ongoing at our institution and additional trials with G3139 in combination with or without chemotherapy have recently been initiated for prostate cancer, breast cancer, and lymphoma.

In summary, treatment of human MCC grown in SCID mice with *bcl-2* antisense oligonucleotides decreased Bcl-2 expression, increased apoptosis, and dramatically reduced the tumor size. Our findings provide evidence that human MCC represents another treatment-resistant tumor where modulation of Bcl-2 expression, using systemic therapy with an antisense oligonucleotide, has potential relevance to clinical therapy.

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